EFFECTS OF OVARIECTOMY OR HYPOPHYSECTOMY ON DAY ONE OF PREGNANCY ON DEVELOPMENT AND TRANSPORT OF FERTILIZED RAT EGGS

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SUMMARY

The development of fertilized eggs and their transport through the oviduct were studied in rats ovariectomized or hypophysectomized on day 1 of pregnancy. When killed on day 5 of pregnancy, 100%, 66% and 27% of the eggs were in the uterus of intact controls, ovariectomized and hypophysectomized rats, respectively. Thus both ovariectomy and hypophysectomy retarded transport of a proportion of the eggs. Ninety-nine per cent, 72% and 78% of the eggs were in the blastocyst stage in intact controls, ovariectomized and hypophysectomized rats. The remainder of the eggs were either morulae or degenerated.

Blastocysts and morulae recovered from the hypophysectomized rats were transferred to the uteri of pseudopregnant recipients: 52–57% developed into normal term foetuses compared to 57% for intact controls.

In hypophysectomized rats killed in the morning of day 6, the number of eggs recovered was greatly reduced as compared with rats killed on day 5. This loss of eggs was prevented by placing a ligature at the cervical end of the uterus in the morning of day 5. It was therefore concluded that the eggs had been expelled from the uterus into the vagina between days 5 and 6.

INTRODUCTION

It has been shown that oestrogen and progesterone can cause acceleration or deceleration of egg passage through the oviduct (Chang & Harper, 1966; Harper, 1966; Greenwald, 1967, 1968; Humphrey, 1968), but little attention has been focused on the effects of ovarian hormones on the development of fertilized eggs. It has been claimed that ovariectomy of mated females shortly after ovulation has effect neither on the development of fertilized eggs to the blastocyst stage nor on their transport through the oviduct in rabbits (Corner, 1928; Adams, 1958) and rats (Alden, 1942), but that it retards both development and transport in mice (Whitney & Burdick, 1939).

Alden (1942) concluded that in the rat "ovariectomy following ovulation does not
prevent development or transport of ova through the oviduct to the uterus". The
weakness of Alden's conclusion was that it was based on a small number of rats.
For example, to determine whether fertilized eggs can develop to the blastocyst
stage, he used three rats which yielded six blastocysts, an undisclosed number of
blastocysts, and no eggs, respectively. The first aim of the present study was to re-
examine Alden's conclusion by using larger numbers. If, in the ovariectomized rats,
the fertilized eggs did develop into blastocysts, then the second aim was to test the
ability of such blastocysts to develop into term foetuses. The third aim was to
determine whether in ovariectomized rats the eggs enter the uterus at the normal
time and whether they are retained in the uterus.

Because of unresolved technical difficulties, ovariectomy on day 1 of pregnancy
(but not on subsequent days) frequently results in injuries to the oviducts. To circum-
vent these difficulties, hypophysectomy instead of ovariectomy was done. For the
purpose of the present investigation, hypophysectomy has the advantage that it
prevents both the ovaries and the adrenals from producing sex hormones. The
possible shortcoming of hypophysectomy is that there may be, and probably is,
a lag period from the time of hypophysectomy until functional ovariectomy is attained.

MATERIALS AND METHODS

The rats used were adult virgin females of the Holtzman strain, weighing 180-
200 g and housed in air-conditioned quarters in which the lights were on from 06.00
20.00 h. The females were caged with fertile males on the day of pro-oestrus and
examined the next morning (= day 1 of pregnancy) for the presence of a vaginal plug
or spermatozoa in the vagina. Mated females were ovariectomized or hypophysec-
tomized between 09.30 and 10.30 h on day 1 of pregnancy. The hypophysectomized
rats were provided with drinking water containing 5% glucose. Completeness of
ovariectomy and hypophysectomy was checked at the time of autopsy. One rat was
discarded because of incomplete hypophysectomy.

Recovery and examination of eggs. The operated rats were killed on day 5 or 6. The
reproductive tract was divided into two parts: oviduct plus about 2 mm of uterus,
and the remainder of the uterus. The right and left oviducts and uteri were flushed
into separate dishes with medium consisting of one part rat plasma and two parts
0.9% NaCl solution. Under a dissecting microscope (30–60 x ) the eggs in the medium
were counted and classified as: degenerated eggs (including both unsegmented and
fragmented eggs), morulae, young blastocysts, and mature blastocysts. The young
blastocyst stage was arbitrarily defined as blastocysts in which the size of the blasto-
coele was not larger than one-quarter of the whole blastocyst. Quite often the
blastocoele was only equivalent to one or two blastomeres in size.

Developmental capacity of eggs. To test the developmental capacity of the eggs
(excluding the obviously degenerated ones) recovered from the hypophysectomized
pregnant rats, they were transferred into the uteri of day 4 pseudopregnant recipi-
ents. Pseudopregnancy was induced by mechanical stimulation of the cervix (De-
Feo, 1966) on the morning of the day of oestrus (= day 1 of pseudopregnancy). Three
to six eggs in approximately 1 µl medium were injected into the uterine lumen with
a 10 µl microsyringe (Hamilton Company, Whittier, California). The recipients were
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killed on day 19 of pseudopregnancy: the numbers of foetuses and resorption sites were counted, and the weights of foetuses recorded for comparison with controls. Whenever morulae and blastocysts were available in sufficient numbers, they were transferred into separate uteri of the same recipient to assess the developmental capacity of each. There was no difference in viability between morulae and blastocysts.

RESULTS

Recovery and examination of eggs

The results are shown in Table 1. In the intact controls (group 1) all eggs were found in the uterus on the morning of day 5; 91% of them were mature blastocysts, and the remainder were young blastocysts. In the ovariectomized rats (group 2), 34% of the eggs were still in the oviduct and 66% in the uterus; 15% of eggs were still in the morula stage, 13% were degenerated and 72% blastocysts. A relatively high percentage of the eggs recovered from the oviduct were morulae (30%) and degenerated eggs (30%), and only 39% were blastocysts (Table 2). In contrast, most of the eggs which had reached the uterus (89%) were blastocysts, and only 7% and 5% respectively were morulae and degenerated eggs. As shown in Table 1,

Table 1. The location and developmental stage of fertilized eggs recovered on days 5 and 6 in rats ovariectomized or hypophysectomized on day 1 of pregnancy

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Day of autopsy</th>
<th>No. of rats</th>
<th>Oviducts</th>
<th>Uterus</th>
<th>Total</th>
<th>Mean* ± S.E.M.</th>
<th>Degenerated</th>
<th>Young blastocyst</th>
<th>Mature blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Intact</td>
<td>5</td>
<td>12 (0)†</td>
<td>0</td>
<td>135</td>
<td>135</td>
<td>11.3 ± 0.54</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>2. Ovariectomy</td>
<td>5</td>
<td>16 (4)</td>
<td>23</td>
<td>44</td>
<td>67</td>
<td>4.2 ± 0.95</td>
<td>9</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>3. Hypophysectomy</td>
<td>5</td>
<td>21 (0)</td>
<td>157</td>
<td>57</td>
<td>214</td>
<td>10.2 ± 0.40</td>
<td>20</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>4. Hypophysectomy</td>
<td>6</td>
<td>18 (5)</td>
<td>2</td>
<td>48</td>
<td>50</td>
<td>2.8 ± 0.74</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5. Hypophysectomy</td>
<td>6</td>
<td>13 (1)</td>
<td>0</td>
<td>114</td>
<td>114</td>
<td>8.8 ± 1.03</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>plus uterine ligature‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Includes animals without eggs.
† Number of rats with no eggs in parentheses.
‡ A uterine ligature was placed at the cervical end of each uterine horn on the morning of day 5.

Table 2. Stage of development of fertilized eggs, as related to their location in the reproductive tract, on day 5 of pregnancy in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Location</th>
<th>Degenerated</th>
<th>Morulae</th>
<th>Young blastocyst</th>
<th>Mature blastocyst</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Ovariectomy</td>
<td>Oviduct</td>
<td>7 (30)</td>
<td>7 (30)</td>
<td>0 (0)</td>
<td>9 (39)</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uterus</td>
<td>2 (5)</td>
<td>3 (7)</td>
<td>4 (9)</td>
<td>35 (166)</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>Hypophysectomy</td>
<td>Oviduct</td>
<td>13 (8)</td>
<td>22 (14)</td>
<td>22 (14)</td>
<td>100 (64)</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uterus</td>
<td>7 (12)</td>
<td>8 (14)</td>
<td>12 (21)</td>
<td>30 (53)</td>
<td>57</td>
</tr>
</tbody>
</table>

The figures in parentheses represent the percentage of the total number of eggs recovered from either the oviduct or the uterus.
not only was the average number of eggs recovered reduced as compared with the intact controls (4.2 v. 11.3), but the number of rats without eggs was also increased (4 out of 16 rats v. none of 12 rats).

All the hypophysectomized rats killed on day 5 had a normal number of eggs (group 3, Table 1): 73% of the eggs were in the oviduct and 27% in the uterus; 9% were degenerated eggs, 14% morulae, 16% young and 61% mature blastocysts. Of the eggs present in the oviduct, 8% were degenerated eggs, 14% morulae, 14% young and 64% mature blastocysts; the corresponding values for eggs in the uterus were 12, 14, 21 and 53%, respectively (Table 2). Thus, hypophysectomy retarded both the transport and the development of some of the eggs. However, the delay in development did not occur more often among eggs retained in the oviduct than in those which reached the uterus on time.

When the hypophysectomized rats were killed on day 6 (group 4, Table 1), five out of 18 animals had no eggs. Moreover, in those with eggs, the mean number recovered (3.9) was greatly reduced. Nearly all the recovered eggs were in the uterus and 86% had reached the mature blastocyst stage. In order to determine whether the reduced number of eggs in group 4 was due to their expulsion from, or cytolysis by, the uterus, the uteri of 13 rats were ligated at the cervical end in the morning of day 5 and autopsied 24 h later (group 5, Table 1). Only one rat in this group had no eggs. In all other animals, the eggs were found in the uteri; the mean number of eggs recovered (8.8) was similar to that of rats killed on day 5 (group 3, Table 1). Of the 114 eggs recovered 101 (88%) were mature blastocysts, 12 (11%) were degenerated and one (1%) was a morula. Apparently the poor recovery rate of eggs in group 4 was due to their expulsion from the uterus.

**Developmental capacity of eggs**

The results are shown in Table 3. Fertilized eggs were recovered on day 5 of pregnancy from intact and hypophysectomized rats (groups 1 and 3, respectively) and were transferred into the uteri of day four pseudopregnant recipients. Fifteen days later the recipients were killed. In the intact group, the 187 eggs transferred yielded 107 (57%) foetuses and 16 (9%) resorption sites (i.e. a site where the embryo was resorbed some time after implantation). In the hypophysectomy group, the 53 eggs transferred yielded 30 (67%) foetuses and 5 (9%) resorption sites.

When the eggs of hypophysectomized rats were recovered on day 6 (group 5) and then transferred, the yield was 52% foetuses and 21% resorption sites. Of the 14

### Table 3. The number of day-19 foetuses resulting from eggs recovered from hypophysectomized donors and transferred to day-4 pseudopregnant recipient rats

<table>
<thead>
<tr>
<th>Donors</th>
<th>Day killed</th>
<th>No. of eggs transferred</th>
<th>No. of recipients</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Intact</td>
<td>5</td>
<td>187</td>
<td>17</td>
</tr>
<tr>
<td>Group 3</td>
<td>Hypophysectomy</td>
<td>5</td>
<td>53</td>
<td>7</td>
</tr>
<tr>
<td>Group 5</td>
<td>Hypophysectomy plus uterine ligation</td>
<td>6</td>
<td>66</td>
<td>8</td>
</tr>
</tbody>
</table>
resorption sites in the last group, five were found in one recipient which, in addition, had two live foetuses out of nine blastocysts transferred. There were no statistically significant differences in the yield of foetuses and resorption sites between the three groups. The foetal weight and morphology in the two hypophysectomy groups were normal.

DISCUSSION

The present results show that after ovariectomy or hypophysectomy on day 1 of pregnancy, the transport and the development of the eggs were retarded. On day 5 of pregnancy all the eggs in the intact rats were in the uterus. In contrast, only 27% and 66% of the eggs were found in the uterus of the hypophysectomized and the ovariectomized rats, respectively. The greater delay of egg transport in the hypophysectomized as compared with the ovariectomized rats could have been due to: (1) lack of stimulation to the adrenals which could be a potential source of ovarian hormones, and (2) impairment of the overall well-being of the animal as reflected by the decreased body weight. For example, the deficiency in thyroid hormones could cause general slow-down of metabolism and decreased muscular activity which could affect egg transport through the oviduct. Our results support those of Nobunaga (1968), who found that in cycling rats ovariectomized shortly after ovulation, some eggs remained in the oviducts up to 100 h after ovulation.

On the morning of day 5, 15% and 14% of eggs were still morulae in the ovariectomized and the hypophysectomized rats, respectively, as compared to only 1% in the intact animals. In the ovariectomized rats there was a relationship between the delay in the transport and the delay in the development of eggs, i.e. those eggs which were in the oviducts had a higher percentage of morulae and degenerated eggs than those which had reached the uterus (Table 2). On the other hand, there did not seem to be a relationship between delay of transport and delay of development in the hypophysectomized rats (Table 2). This difference between the ovariectomized and hypophysectomized groups may be related to surgical trauma inflicted on the oviduct during ovariectomy.

In the hypophysectomized rats killed on day 5 of pregnancy, 14% of the recovered eggs were still morulae when they should have been blastocysts. However, this delay in development did not have any further deleterious effects, as it could be shown that such morulae retained their potential to develop into term foetuses.

The effects of hypophysectomy on day 1 of pregnancy on the recovery and the development of fertilized eggs have been reported for rats (Mayer, 1966) and mice (Bindon, 1969). In pregnant rats hypophysectomized at 18.00 h of day 1 (approximately 8 h later than in the present study), blastocysts and some morulae were recovered from days 5 to 8 (Mayer, 1966). However, the results were highly variable in terms of the proportion of animals with eggs and the number of eggs recovered per animal, which made a quantitative assessment difficult. In mice hypophysectomized at 10.00 h on day 1, the development of some eggs was retarded, and the number of eggs recovered per animal on days 4–8 was greatly reduced (Bindon, 1969). Transport through the oviduct and the developmental potential of eggs were not studied by either Mayer (1966) or Bindon (1969).

In the present study, as a result of hypophysectomy, most of the eggs were expelled
from the uterus between the mornings of days 5 and 6. This expulsion was unexpected since spontaneous contraction of the uterus is dependent on oestrogen (Frank, Bonham & Gustavson, 1925; Csapo, 1950, 1956; Csapo & Corner, 1953), and presumably the ovary does not put out any oestrogen in the absence of gonadotrophic hormone stimulation. To explain this apparent paradox, we postulate the following. During pro-oestrus and oestrus, oestrogen is secreted by the ovary and the uterus becomes oestrogen-dominated. After rupture of the follicles, corpora lutea form and begin to secrete increasing amounts of progesterone. However, hypophysectomy on day 1 deprives the corpora lutea of luteotrophin, so that even if some progesterone is produced the level is insufficient to override the effect of oestrogen on the uterus; this effect is retained at least until the time the eggs are expelled. The concept of a residual oestrogen effect is supported by the results of other investigators. In spayed does, the oestrogen pattern of uterine contraction was still evident 96 h after oestrogen injection (Csapo & Corner, 1952). Adams (1965) found that, if the number of corpora lutea (‘freshly ruptured follicles’) in rabbits was adjusted to two or less by enucleation or removal of one or both ovaries 14–18 h post coitum, the majority of eggs were expelled into the vagina. He attributed this to a residual oestrogen effect on the uterus.

From the present results, it is concluded that development of fertilized rat eggs, from the one-cell stage to the blastocyst stage, can take place in the absence of stimulation by the ovarian hormones. However, the hormonal deprivation affects some of the eggs which either degenerate or develop at a slower pace. We cannot exclude the possibility of residual effects of the ovarian hormones. Using a different experimental approach, Dickmann (1969) showed that transformation of morula to blastocyst can occur in the absence of ovarian hormones; in this study the possibility of residual effects of ovarian hormones was excluded. It should be kept in mind that the ovarian hormones can affect pre-implantation development; practically all morulae degenerated in ovariectomized, progesterone (2 mg/day)-treated rats (Dickmann, 1970).

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REFERENCES

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